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Experiment report

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Structural modifications of crystalline polysaccharides in supercritical carbon dioxide

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1. Aim of the experiments

Originally, as described in proposal 02-01-670, our project aimed at using wide-angle X-ray scattering (WAXS) to study *in situ* the structural modifications occurring in crystalline (cellulose, chitin) and semi-crystalline (starch) polysaccharides in $scCO_2$. As explained in the following, during preliminary experiments performed on the French CRG D2AM beamline, no significant swelling or destructuring effect was detected, suggesting that CO_2 alone was inert for the specimens. On the other hand, we realized that pressure and thermal properties of polysaccharide crystals, as well as allomorphic transitions could be dynamically monitored using WAXS. As a consequence, two directions were defined for the experiments conducted during the allowed 9 shifts of 8 h. In a first group of experiments, cellulose acetylation was performed in scCO2 replacing toluene that is classically used as a non solvent of cellulose. The structural modifications were monitored *in situ*. A second group focused on the phase transitions occurring in hydrated or wet crystalline polysaccharides heated in pressurized environment.

2. Experimentals

Material. Cellulose whiskers were prepared by acid-hydrolyzing cotton fibers as well as microfibrils from *Halocynthia*, a sea animal also called tunicate, and *Glaucocystis*, an alga. Ramie fibers were also used. Cotton microcrystals were used as a powder (**Fig. 1a**). *Halocynthia* and *Glaucocystis* whiskers were mixed with poly(vinyl-alcohol) (PVA) and the resulting gel was repeatedly extended to obtain oriented specimens. After drying, the PVA matrix was dissolved and rigid fibers containing only cellulose were obtained (**Fig. 1b**). Cellulose triacetate (CTA) specimens were prepared by acetylation of cellulose artificial fibers. The specimens were soaked for 3 h at room temperature in a toluene:acetic anhydride (50:50) solution containing a catalytic amount of HClO and washed with ethanol. Chitin fibers were obtained by orienting the microfibrils excreted by diatoms using the PVA method previously described. Native "potato amylopectin" starch (PAPS) granules were used as a powder, hydrated in a 95% r.h. (relative humidity) atmosphere during a few days (**Fig. 1d**).

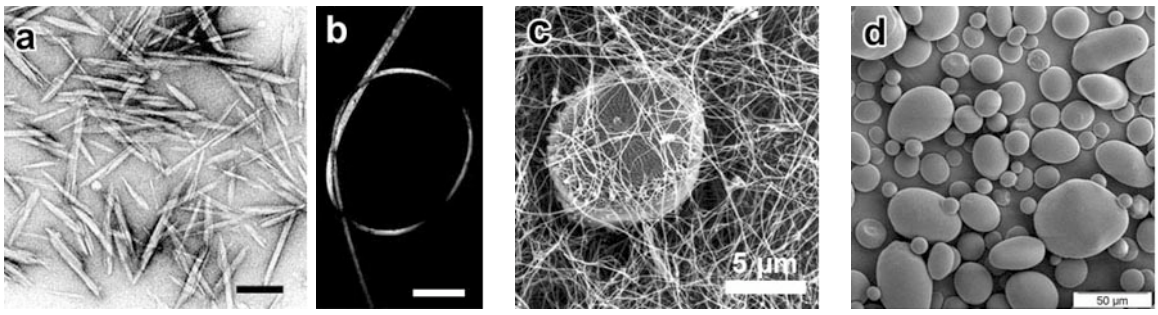

Figure 1. a) Cotton cellulose whiskers (TEM image – bar : 100 nm) ; **b**) oriented fiber containing *Halocynthia* cellulose whiskers (bar : 5 mm) ; **c**) diatom excreting chitin spines (SEM image) ; **d**) potato amylopectin native starch granules (SEM image).

Experimental set-up. A stainless steel specimen holder was specifically designed for our experiments in order to hold fibrillar or powder samples in a glass capillary while having liquid in a surrounding container (**Fig.2**). Cellulose, in the form of powder or oriented fibers, as well as artificial chitin fibers were inserted into 1 mm-wide glass capillaries (**Fig. 2a**). In some cases, the sample was soaked in a liquid (**Figs. 2b,c**). The surrounding container was also filled with liquid. Hydrated starch powders were put into glass capillaries with liquid water in the separate container (**Fig. 2d**).

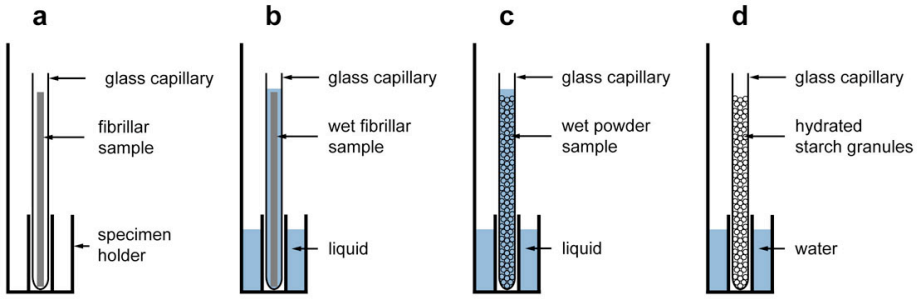

Figure 2. Sample set-up in the case of fibrillar (**a,b**) and powder specimens (**c,d**). The height of the specimen holder is about 2 cm.

The specimen holder was inserted in a sapphire tube (**Fig. 3a**) and placed inside the cell described in Testemale *et al.*, *Rev. Sci. Inst.* **76** (2005), 43905 (**Fig. 3b**). Depending on the experiment, the chamber was pressurized using CO_2 or He, at pressures varying from 10 to 100 bars. The samples were then heated at temperatures in a 25-300°C range. The details about the heat-pressure treatments are given for each sample in the Results section. The samples were probed by a X-ray beam at a 24 keV energy. WAXS patterns were recorded using a CCD detector during 20, 60 or 200 s exposure times, with an angular range of ±11° defined by the exit window of the pressure cell (**Fig. 3c**).

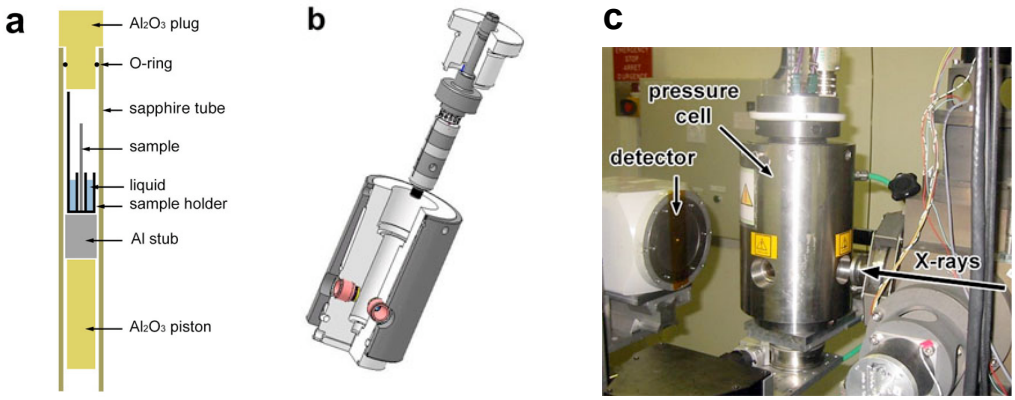

Figure 3. a) sample set-up in the sapphire cell ; **b**) scheme and **c**) picture of the pressure cell.

3. Results and discussion

3.1. Preliminary experiments (January 2005)

Preliminary experiments were carried out to test the experimental set-up and study the behavior of cellulose and starch in $scCO_2$. Pieces of artificial cellulose fibers were put in a glass capillary. The CO_2 pressure was set to 100 bars and the temperature slowly increased to 200°C. Sharp reflections of native cellulose I were observed and a shift of the peaks due to the thermal expansion of the lattice was detected. It was shown that the distortions introduced by the detector should be corrected with care to get an accurate value of the expansion coefficient. Hydrated PAPS starch granules were heated up to 200°C at 10°C/min, at a CO_2 pressure of 100 bars, until the diffraction pattern disappeared. Below 50°C, no significant difference was detected in the patterns recorded in gaseous, liquid and $scCO_2$. Initial hexagonal B-type was recognized. The intensity remained rather constant between 25 and 80°C, with a slow shift of the stronger peaks to lower angles indicating thermal dilatation. The peaks rapidly decreased from 80°C and around 130°C, as the intensity strongly decreased, the residual peaks shifted to larger angles. Despite the very low intensity, the pattern was unambiguously assigned to monoclinic A-type, meaning that a phase transition occurred before decrystallization of starch.

SEM and polarized optical microscopy images show that the starch granules located at the open part of the capillary were preserved (**Figs. 4a,b**), considering the high temperature reached during the experiment (200°C). We thus assumed that the contact with CO_2 and the heating dehydrated starch to the extent that no significant degradation was observed. On the contrary, the granules in the lower part of the capillary were transformed into a glassy alveolar opaque material (**Fig. 4c**), suggesting that enough humidity remained and that extensive degradation occurred. Lastly, in the region probed by the beam, the material was hard and transparent (**Fig. 4a**), meaning that the X-ray beam also had an impact on starch degradation. *We thus concluded that supercritical CO_2 alone did not swell polysaccharide crystals. Sample degradation and changes in unit cell parameters were only ascribed to the effects of temperature and pressure and, in the case of starch, hydration level.*

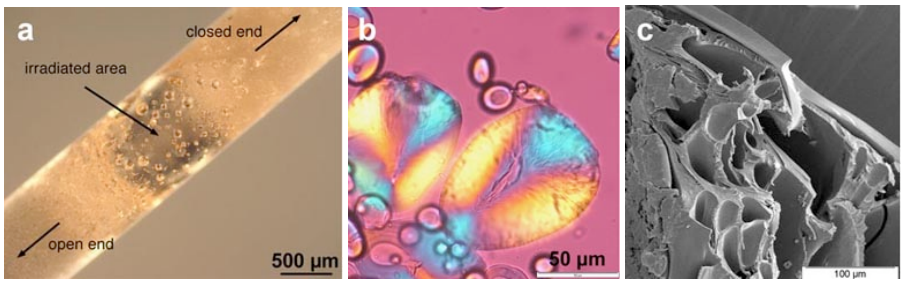

Figure 4. PAPS granules retrieved from the capillary after treatment in $scCO_2$: **a**) view of the capillary; **b**) granules taken near the open end observed by polarized light microscopy; **c**) granules taken near the closed end (SEM image).

3.2. Acetylation of cellulose in supercritical CO_2

Powders of cellulose microcrystals or pieces of fibers containing oriented cellulose whiskers were put in a glass capillary and soaked in acetic acid. The external container of the specimen holder was filled with a few drops of acetic anhydride (Ac_2O) and a catalytic amount of H_2SO_4. The CO_2 pressure was set to 90 bars and the temperature slowly increased. Diffraction patterns were recorded every 10 or 20°C. Apart from slight shifts due to thermal expansion, the patterns recorded on cotton whiskers (**Fig. 5a**) and a ramie fiber (**Fig. 5b**) did not change until around 100°C. Then, the pattern of CTA II was observed in both cases. Conventional fibrous acetylation using a mixture of toluene and acetic acid proceed at room temperature and results in CTA I with minimum morphological change. In this experiment, we could not record patterns during the transition as the reaction appeared to be heterogeneous and we had to probe different regions to find transformed areas. The diffusion of the reacting Ac_2O or the catalyzing H_2SO_4 was much hindered than expected in the system and the reaction was heterogeneous inside capillary, which makes the interpretation difficult.

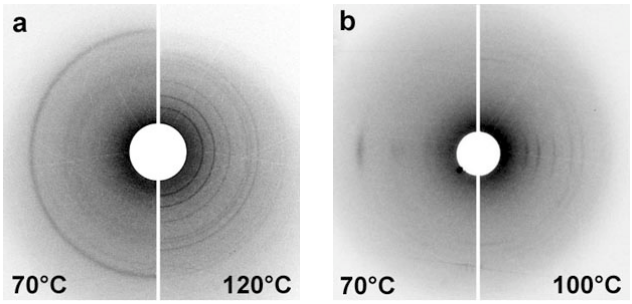

Figure 5. Acetylation of native cellulose I : **a**) powder diagrams from cotton whiskers at 70°C *(left)* and 120°C, after transformation into CTA II *(right)* ; **b**) fiber diagrams from a ramie fiber at 70°C *(left)* and 100°C, after transformation into cellulose triacetate II *(right).*

The CTA I to CTA II transition was observed in a CTA I fiber that was heated to 300°C in $scCO_2$ only. Swelling of the crystal and thermal expansion were detected (**Fig. 6a**). Between 290° and 300°C, fusion of the CTA I specimen occurred. After finding a new area of interest, the observed powder pattern indicated that the sample had recrystallized into CTA II (**Fig. 6b**).

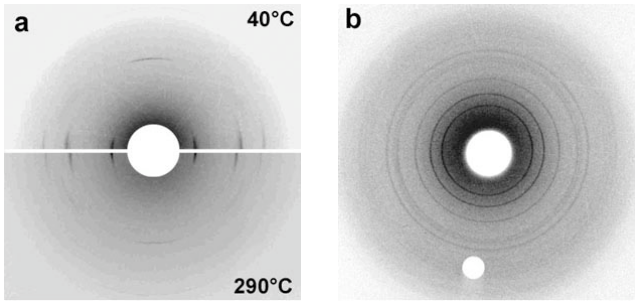

Figure 6. Transition from CTA I to CTA II in $scCO_2$: **a**) fiber patterns at 40°C and 290°C ; **b**) powder pattern of CTA II illustrating the transition that took place after fusion of the material, recrystallization and return to room temperature.

3.3. Annealing of cellulose triacetate I in He

CTA I samples prepared by acetylating tunicate fibers were heated to 250°C in He at 10 bars. The patterns shown in **Fig. 7** clearly show the effect of annealing on the crystallinity of the specimen. A very nice pattern appeared in a matter of minutes within a temperature range of 40°C. Surprisingly, in the annealing treatments mentioned in literature, the samples are heated for several hours.

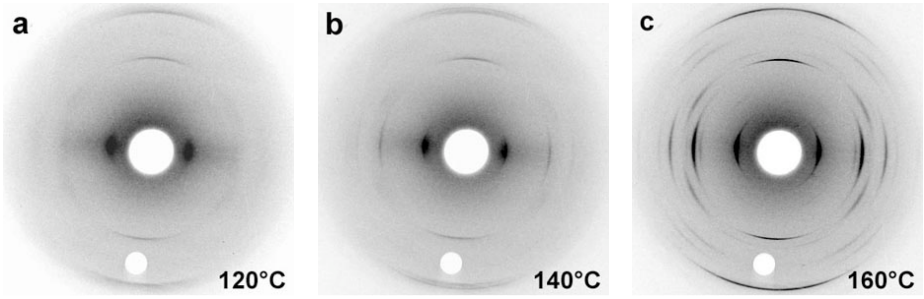

Figure 7. Annealing of CTA I in He : **a,b,c**) fiber patterns recorded at 120,140 and 160°C, respectively.

3.4. Phase transitions in pressurized He

3.4.1. Cellulose and chitin

Native cellulose I contains two allomorphs with parallel chains, namely $I\alpha$ and $I\beta$. We heated a fiber of $I\alpha$-rich *Glaucocystis* whiskers up to 280°C, in He at 90 bars. The $I\alpha$ to $I\beta$ transition was detected from about 200°C, mostly through the appearance of one additional meridional reflection (**Fig. 8a**). Chitin fibers made of diatom spines soaked in water were heated up to 200°C, in He at 90 bars. The temperature was then decreased to 25°C and increased again to 260°C. The initial pattern suggests that the sample is a mixture of two types of chitin hydrate. A first transition occurred at around 85°C and a second at about 105°C. The phase did not change to 200°C. Contrary to the transitions observed in cellulose I, CTA and, further on, starch, those detected on chitin were reversible. However, a hysteresis was observed and upon cooling, a unique transition occurred at about 50°C. When the sample was heated up again, the transitions at 85 and 105°C took place again.

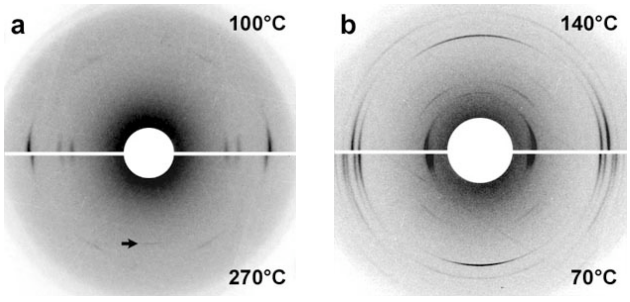

Figure 8. a) $I\alpha$ *(top)* to $I\beta$ *(bottom)* transition in a *Glaucocystis* cellulose fiber heated to 270°C. The arrow indicates an additional reflection from $I\beta$; **b**) two hydrated forms of diatom β-chitin respectively formed by heating to 140°C then cooling to 70°C.

3.4.2. Starch

Experiments on PAPS granules were performed at 16 bars in He pressures with liquid water in the external container. The hydrated powder was heated to 120°C at a rate of about 2.5°C/min. As shown in **Fig. 9a**, the profile of the native B-type is clearly recognized up to 100°C. There is a slight shift to lower *q*-values due to thermal expansion. From 100 to 120°C, the intensity decreases and some peaks shift to larger angles. During the return to room temperature, the intensity increases again and a new pattern corresponding to A-type appears. A comparison between the powder patterns before and after the phase transition is shown in **Fig. 9b**. SEM images of the starch granules that were submitted to the heat-pressure treatment show no change of general aspect. The birefringence pattern seen with polarized optical microscopy is similar to that of native granules. The molecular orientation is preserved and no swelling associated to gelatinization is detected.

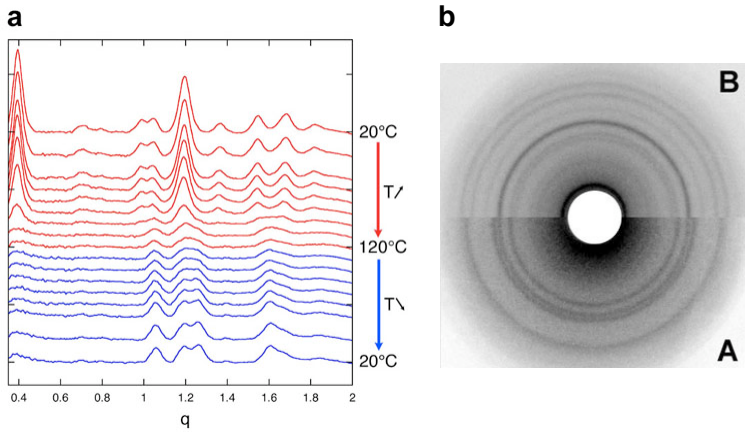

Figure 9. B→A phase transition in PAPS starch granules (He 16 bars). The sample was heated up to 120°C. **a**) Diffraction profiles at different temperatures after subtraction of the diffusion background from He, sapphire cell and amorphous starch. B-type and A-type profiles are recognized at 20°C in the initial *(top)* and final *(bottom)* spectra. **b**) Powder patterns of initial B-type *(top half)* and final A-type *(lower half)* at 20°C.

The B→A transition in starch granules is not a new result. It has been described by various authors and is used at the industrial scale to modify the functional properties of starch. However, the process takes 24 h. In our case, the transition was rather fast, occurring in a matter of minutes. This suggests that pressure has an influence on the heat-moisture treatment of starch. The fact that we successfully performed the B→A transition validates our experimental set-up. It seems that the initial 35% moisture that is necessary for the treatment did not significantly change throughout the pressure/heat treatment, thanks to the small quantity of liquid water placed in the container surrounding the capillary.

4. Conclusion and perspectives

Supercritical CO_2 alone appears to be inert with respect to crystalline polysaccharides. However, chemical reactions are possible when reagents are used in conjunction with $scCO_2$. The structural transitions associated with the acetylation of native cellulose whiskers in $scCO_2$ have been successfully monitored by WAXS in the pressure cell but a better control in mixing the reagents is desirable. It should be possible to perform other types of chemical reactions provided that the chemicals are inert with respect to the materials constituting the cell or that a new cell is designed using resistant materials.

The feasibility to follow crystalline transformations *in situ* using highly crystalline oriented polysaccharide specimens has been demonstrated for cellulose, chitin and starch. There are several other known solid-state transitions and a systematic investigation combining the use of the pressure cell for hydrothermal treatments and synchrotron WAXS analysis should provide substantial and important knowledge on the molecular mechanism of the transformations in polysaccharide crystals.